

# The *Aspergillus* Genome Database (AspGD): recent developments in comprehensive multispecies curation, comparative genomics and community resources

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## ABSTRACT

The *Aspergillus* Genome Database (AspGD; <http://www.aspgd.org>) is a freely available, web-based resource for researchers studying fungi of the genus *Aspergillus*, which includes organisms of clinical, agricultural and industrial importance. AspGD curators have now completed comprehensive review of the entire published literature about *Aspergillus nidulans* and *Aspergillus fumigatus*, and this annotation is provided with streamlined, ortholog-based navigation of the multispecies information. AspGD facilitates comparative genomics by providing a full-featured genomics viewer, as well as matched and standardized sets of genomic information for the sequenced aspergilli. AspGD also provides resources to foster interaction and dissemination of community information and resources. We welcome and encourage feedback at [aspergillus-curator@lists.stanford.edu](mailto:aspergillus-curator@lists.stanford.edu).

## INTRODUCTION

The *Aspergillus* Genome Database (AspGD) is a unique web-based resource focusing on a single genus with a dense representation of sequenced and publicly available whole-genome sequences. AspGD's mission is to serve the scientific research community by improving the structural and functional annotation associated with the *Aspergillus* genomes and providing a web-based platform for interrogating the similarities and differences between strains

and species. The database currently houses 10 genomes representing eight distinct species and, with third-generation sequencing technologies making sequencing more accessible, we expect that number to increase markedly over the next year. As more genomic data become available, there is a concomitant increase in the power of the comparative platform for exploring the diversity of the aspergilli, which inhabit diverse environmental niches and include a pathogen of humans, agriculturally devastating toxin-producers and species with utility in industry and in the laboratory.

A hallmark of AspGD is the expert literature-based curation of the gene, protein and sequence data for key *Aspergillus* species. Species were prioritized for curation in consultation with the *Aspergillus* Genome Research Policy Committee (AGRPC), as well as the larger *Aspergillus* research community at their annual meetings. The first species targeted for curation was *Aspergillus nidulans*, an important model organism and the best characterized species with the largest body of literature (1). The next species chosen was *Aspergillus fumigatus*, the main cause of aspergillosis, an invasive fungal infection affecting immune-compromised patients, due to its clinical relevance (2,3). We are currently curating *Aspergillus niger*, which is of great importance to industrial applications involving enzyme production (4,5).

### Comprehensive curation and annotation of *A. nidulans* and *A. fumigatus* genes

AspGD now features comprehensive curation and annotation of the genomic catalog of two entire *Aspergillus*

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species, *A. nidulans* and *A. fumigatus*. AspGD curators have read the entire available published scientific literature describing genes in both organisms. From this corpus, we have collected gene names, including all of the alternate names (aliases) that have been published for each gene; annotations describing the gene product function, cellular role and localization, with the controlled vocabularies provided by Gene Ontology (GO) (6); mutant phenotypes and lists of all published references concerning each gene. To augment the literature-based annotation, we have inferred additional GO annotations using orthology to characterize genes in other aspergilli (at AspGD) and in *Saccharomyces cerevisiae* (at the *Saccharomyces* Genome Database, SGD, [www.yeastgenome.org](http://www.yeastgenome.org)), as well as the presence of characterized InterPro protein domains and motifs (7). We also use tRNAscan software to generate consistent catalogs of tRNA gene model annotations for each genome in AspGD (8). Current AspGD curation statistics are summarized in Table 1. We are now in the process of adding *A. niger* information into AspGD, and will be expanding the scope of AspGD literature curation to cover additional species in the future, including *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus clavatus*, *Aspergillus terreus* and *Neosartorya fischeri*/*Aspergillus fischerianus*. The *A. fumigatus* genome statistics, like those for the *A. nidulans* genome, are now summarized in a Genome Snapshot page on the AspGD web site, which displays tabular and graphical summaries of gene statistics and annotation and is updated daily to reflect the most up-to-date state of the characterization of the genome and gene catalog.

The information for each gene is displayed on the AspGD web site and also available in downloadable files. We can provide custom-format data files upon request; to request a file, contact us at <http://www.aspergillusgenome.org/cgi-bin/suggestion>. The web site is organized around Locus Summary pages (Figure 1) that list the basic information about each gene and link to additional tabbed pages that provide more extensive information, such as

the history of sequence and major annotation changes (Locus History), the full citation list (Literature Guide), the gene product annotation and supporting evidence (GO) and specifics of each mutant phenotype with details including specifics of the experimental conditions, observations and strain background. Recently, we have implemented an additional Protein Details page for each protein-coding gene. The protein details shown include a graphical display of protein domain organization, the molecular structure of the top hit in the Protein Data Bank (PDB) (9), the protein sequence, the set of predicted physicochemical properties and links to tabular enumerations of all unique domains and domains shared with other proteins in the genome, transmembrane segments and signal peptides, plus other domain exploration tools.

Accommodation of the new *A. fumigatus* information in AspGD alongside the *A. nidulans* data required numerous extensive updates to the web site and the database structure. In the new, multispecies format, we have interconnected the Locus Summary pages between species via orthology relationships. The Locus Summary page for a *A. nidulans* gene is connected to the Locus Summary page for its ortholog in *A. fumigatus*, and vice versa, by hyperlinks displayed in the section labeled 'Orthologous genes in *Aspergillus* species', located near the top of the page, making it easy to navigate between orthologs (Figure 1). The link labeled 'View ortholog cluster' displays the entire gene cluster in the Sybil comparative genomics browser, which is described in more detail below.

All of the tools on the AspGD web site have been updated to handle multispecies information. Tools such as Quick Search, which queries data from multiple species, feature a redesigned results page (Figure 2A) with sections on the page for results that are specific to each species and a separate section for results that are not species specific (e.g. GO terms, authors and reference information, colleagues). Tools that query sequence-based data now provide a species selection option (Figure 2B),

**Table 1.** AspGD curation statistics

	<i>Aspergillus nidulans</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>
Number of ORFs	11 286	9887	14 071
Number of tRNAs	222	178	264
Manual GO annotations	20 233	16 994	N/A
Features with manual GO annotations	8781	7786	N/A
Orthology-based GO annotations	8295	9220	9637
Features with orthology-based GO annotations	1973	2229	2342
Protein-domain (InterPro)-based GO annotations	14 505	13 068	16 614
Features with protein-domain (InterPro)-based GO annotations	5748	5226	6348
Features with orthology-based description lines	5016	5430	N/A

AspGD curation statistics were determined as of 1 September 2011. The number of Open Reading Frames (ORFs) includes characterized (verified) and uncharacterized genes that are predicted to encode protein products. The tRNA complement was predicted at AspGD using tRNAscan-SE (8). GO annotations have been assigned based on manual curation of the scientific literature, predicted based on orthology to characterized gene products from *S. cerevisiae* or other aspergilli, and predicted based on characterized InterPro protein domains (7). Manual curation of the scientific literature about *A. nidulans* and *A. fumigatus* is complete and up-to-date; curation of *A. niger* is commencing in September 2011. In species for which manual curation is complete, predicted gene products that are uncharacterized, but which have orthologs, are given descriptions containing orthology-based information.

**AspGD**

Quick Search:  Submit Site Map | Search Options | Help | Contact AspGD | Home

Community Info Submit Data BLAST Primers PatMatch Gene/Seq Resources Advanced Search

**A. fumigatus fre2/Afu1g17270 Summary** Help

**A** Summary Locus History Literature Gene Ontology Phenotype Protein

**B** **fre2 BASIC INFORMATION** [View References]

Standard Name	fre2 <sup>1</sup>
Systematic Name	Afu1g17270
Alias	freB <sup>2</sup> AFUA_1G17270
Feature Type	ORF, Verified
Description	Metalloreductase involved in response to iron starvation; repressed by iron through SreA-dependent regulatory system (2, 3)
Ortholog(s)	<i>S. cerevisiae</i> (FRE3, FRE2)
Orthologous genes in Aspergillus species	<i>A. nidulans</i> FGSC A4 ortholog(s) : AN6400 <i>A. niger</i> CBS 513.88 ortholog(s) : An15g05500, An11g00220, An16g01150 View ortholog cluster : 13 genes among 10 Aspergillus-related species/strains

**C** **GO Annotations**

Molecular Function	View all fre2 GO evidence and references
Manually curated	ferric-chelate reductase activity (IEA with EBI: IPR013130)
Computational	electron carrier activity (IEA with EBI: IPR013130) ferric-chelate reductase activity (IEA with <i>S. cerevisiae</i> : FRE2) flavin adenine dinucleotide binding (IEA with EBI: IPR013130) iron ion binding (IEA with EBI: IPR013130)
Biological Process	cellular iron ion homeostasis (IGI with <i>A. fumigatus</i> : sreA) cellular response to iron ion starvation (IMP) copper ion import (IEA with <i>S. cerevisiae</i> : FRE2) oxidation-reduction process (IEA with EBI: IPR017927) siderophore transport (IEA with <i>S. cerevisiae</i> : FRE3)
Manually curated	
Computational	
Cellular Component	integral to membrane (IEA with EBI: IPR013130) plasma membrane (IEA with <i>S. cerevisiae</i> : FRE2, <i>S. cerevisiae</i> : FRE3)
Computational	

**D** **Mutant Phenotype**

Classical genetics	null
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**E** **Sequence Information**

Last Update	Chr1_A_fumigatus_Af293:4722341 to 4719818   GBrowse												
Subfeature Details	Note: this feature is encoded on the Crick strand. Coordinates: 2011-01-07   Sequence: 2011-01-07												
	<table border="1"> <thead> <tr><th>Relative Coordinates</th><th>Chromosomal Coordinates</th><th>Most Recent Update</th></tr> </thead> <tbody> <tr><td>CDS</td><td>1 to 432 4,722,341 to 4,721,910</td><td>2011-01-07 2011-01-07</td></tr> <tr><td>Intron</td><td>433 to 481 4,721,909 to 4,721,861</td><td>2011-01-07 2011-01-07</td></tr> <tr><td>CDS</td><td>482 to 522 4,721,860 to 4,721,820</td><td>2011-01-07 2011-01-07</td></tr> </tbody> </table>	Relative Coordinates	Chromosomal Coordinates	Most Recent Update	CDS	1 to 432 4,722,341 to 4,721,910	2011-01-07 2011-01-07	Intron	433 to 481 4,721,909 to 4,721,861	2011-01-07 2011-01-07	CDS	482 to 522 4,721,860 to 4,721,820	2011-01-07 2011-01-07
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	<a href="#">Genomic DNA (excluding UTRs)</a> <a href="#">Get Sequence</a>												

**F** **External Links** CADRE Primary AspGDID ASPL0000102792

**ADDITIONAL INFORMATION for fre2**

**Locus History** Gene/Sequence Resources

**LOCUS SUMMARY NOTES for fre2**  
Name listed in the Broad Institute genome\_summary\_per\_gene\_file: ferric-chelate reductase (Fre2), putative

**LAST UPDATED:** 2010-08-25

**REFERENCES CITED ON THIS PAGE** [View Complete Literature Guide for fre2]

- 1) Liu H, et al. (2010) Aspergillus fumigatus AcmU regulates both iron acquisition and gluconeogenesis. *Mol Microbiol* 78(4):1038-54
- 2) Blatzer M, et al. (2011) The metalloreductase FreB is involved in adaptation of Aspergillus fumigatus to iron starvation. *Fungal Genet Biol*
- 3) Schrettl M, et al. (2008) SreA-mediated iron regulation in Aspergillus fumigatus. *Mol Microbiol* 70(1):27-43

**Figure 1.** Locus Summary page for *A. fumigatus* fre2. (A) Navigation tabs lead to expanded reports on Locus History, relevant literature, GO and phenotype annotations and protein details. (B) Basic Information section provides gene names and aliases, a headline-like description of the gene product, links to orthologous genes in other *Aspergillus* species in AspGD and links to orthologs in *S. cerevisiae* at SGD. (C) GO section lists functional annotations derived from manual curation of available data and from orthology-based and protein domain-based computational analysis. (D) Mutant Phenotype section outlines literature-derived experimental data for mutant phenotypes. (E) Sequence Information section gives an overview of the gene model and provides access to sequences (parts of this section have been removed from the figure to conserve space). (F) Additional Information section includes other notes and the list of references cited on the Locus Summary page. The comprehensive citation list for the gene is found on the gene's Literature Guide, accessible from the Literature tab near the top of the Locus Summary page.

**A**

**General Search Results for : fre**

- 19 Gene Ontology terms (GO terms, synonyms)
- 0 Colleagues (by last name)
- 38 Authors (by last name, first initial)
- 0 PubMed ID
- 0 Gene Ontology ID

**Aspergillus nidulans Search Results for : fre**

- 1 Gene names (gene name/alias/ORF name)
- 3 Locus Summary Notes
- 5 General Descriptions
- 7 Phenotypes [Expanded Phenotype Search]
- 11 Ortholog or Best Hit

**Aspergillus fumigatus Search Results for : fre**

- 1 Gene names (gene name/alias/ORF name)
- 2 Locus Summary Notes
- 6 General Descriptions
- 0 Phenotypes [Expanded Phenotype Search]
- 19 Ortholog or Best Hit

**Aspergillus niger Search Results for : fre**

- 1 Gene names (gene name/alias/ORF name)
- 58 Locus Summary Notes
- 0 General Descriptions
- 0 Phenotypes [Expanded Phenotype Search]
- 14 Ortholog or Best Hit

**B**

1. Enter a Name  
or the first few characters followed by \*:  
fre2  
and Select *A. nidulans* FGSC A4  
Examples *A. fumigatus* AF293  
*Candida krusei* CBS 513.88  
ORF - AN10512  
AspGID - ASPL0000078296

If available, add flanking basepairs upstream \_\_\_\_\_ and downstream \_\_\_\_\_  
 Use the reverse complement

2a. Pick a chromosome:  
*A. nidulans* FGSC A4   
 Then enter coordinates (optional):  
to \_\_\_\_\_  
 Use the reverse complement  
The first 100,000 bases of the chromosome or coding sequence will be retrieved if no coordinates are entered.

OR

2b. Upload a file of chromosome regions to download genomic sequences in batch:  
 Choose file / no file selected  
 Use the reverse complement  
Chromosome regions should be specified with the following separated columns (coordinates are optional):  
(i) chromosome, (ii) start\_coordinate, (iii) stop\_coordinate  
Note: All regions in the uploaded file must be for the same strain.

*A. nidulans* FGSC A4 example:  
ChrII\_A\_nidulans\_FGSC\_A4 43123 64321  
ChrIV\_A\_nidulans\_FGSC\_A4 1234 2345  
ChrV\_A\_nidulans\_FGSC\_A4 1432 2412  
mt188\_A\_nidulans\_FGSC\_A4 1432 2412

*A. fumigatus* AF293 example:  
Chr2\_A\_fumigatus\_AF293 4323 6443  
Chr3\_A\_fumigatus\_AF293 641 1343  
Chr1\_A\_fumigatus\_AF293 24278 28745

3. Type or Paste a DNA Sequencer  
The sequence MUST be provided in RAW format, no comments (numbers are okay).  
 Use the reverse complement

**C**

Advanced Search:

Step 1: Select strain (REQUIRED)  
Select a strain to limit search results  
*Aspergillus fumigatus* AF293   
*Aspergillus nidulans* FGSC A4   
*Aspergillus niger* CBS 513.88

Step 2: Select chromosomal feature (REQUIRED)  
Select one or more feature types  
 ORF  
 gene locus  
 rRNA  
 not in systematic sequence  
 pseudogene  
 tRNA  
 repeat\_region  
 ncRNA  
 rRNA  
 Select all chromosomal features

Step 3: Narrow results (OPTIONAL)  
Select search criteria to return specific types of genes. Results will match all selected criteria.  
Select search criteria by clicking on a checkbox, filling in a dialog box, or selecting a menu option.  
Select or unselect multiple options for Chromosomes and GO terms by pressing the Control (PC) or Command (Mac) key while clicking.

Annotation/sequence properties:  
Is a feature that is  
 Uncharacterized  Verified  not in systematic sequence  not physically mapped  transposable element  
AND  
Has introns (excluding UTR introns)  Yes  No  
AND  
Has annotated UTR region(s)  Yes  No  
AND  
Has introns in UTR region(s)  Yes  No  
AND  
Is on the following chromosome sequence(s):  
*Chr1\_A\_fumigatus\_AF293*  
*Chr2\_A\_fumigatus\_AF293*  
*Chr3\_A\_fumigatus\_AF293*  
*Chr4\_A\_fumigatus\_AF293*  
*Chr5\_A\_fumigatus\_AF293*  
*Chr6\_A\_fumigatus\_AF293*  
*Chr7\_A\_fumigatus\_AF293*  
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**Figure 3.** Search and visualization tools in the Sybil comparative genomics module. (A) Synteny gradient indicating the location of homologous blocks relative to a reference sequence, in this example *A. nidulans* FGSC A4. Vertical bars with the same shade of color represent orthologous genes across genomes. (B) Ortholog/Paralog cluster search tool, that allows researchers to retrieve homolog clusters meeting user-defined criteria regarding strain or species distribution. (C) Genomic region comparison depicting the structural annotation of syntenic and non-syntenic genes in a homologous genomic region. The trapezoids connect orthologous genes. (D) Whole-genome display showing the gene density and GC content along the sequence of *A. nidulans* chromosome IV and *A. fumigatus* chromosome 8. Colored vertical bars indicate gene locations.

513.88, *A. oryzae* RIB40/ATCC 42149, *A. terreus* NIH2624 and *N. fischeri* NRRL 181.

### Improvement of gene structure

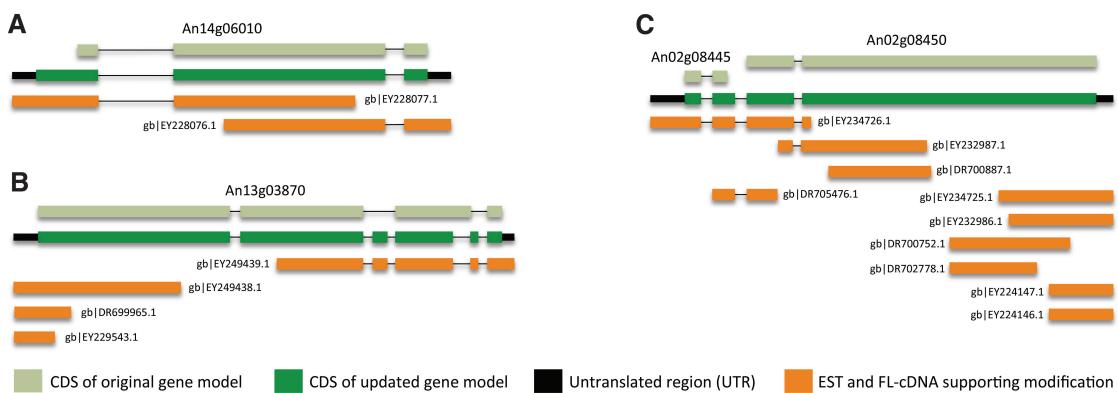
As transcriptome data for the species and strains with genomic sequences stored in AspGD become available, we are mapping and incorporating these data into the structural annotation of those genomes. This effort will progressively refine the current gene models and their products, further facilitating comparative analyses and molecular or biochemical experiments that depend on this information.

To begin, we have performed a set of gene structure modifications on the genome of *A. niger* CBS 513.88 through incorporation of publicly available experimental data comprising *A. niger* expressed sequence tags (ESTs) and full-length cDNAs (12–17). The analysis was performed by PASA (Program to Assemble Spliced Alignments) (18), which automatically redefines intron-exon boundaries, extends UTRs and identifies possible novel isoforms. The modifications performed by PASA are supported by mapping and clustering of transcriptome data, assembly of the aligned sequence data and

comparison with the current structural annotation of the genomes analyzed (Figure 4). The PASA analysis performed on *A. niger* CBS 513.88 resulted in the modification of 4230 genes (30% of the total gene set): the UTRs of 3452 genes were extended, coding sequence alterations were made for 308 genes, the internal exon structure of 719 genes was modified and 15 pairs of genes were merged into single loci. Some genes were subject to multiple types of modification. As more species are added to AspGD, and more experimental transcriptome data become available for *Aspergillus* species, including RNA-Seq, this PASA pipeline will be used for iterative improvements of all of these reference genome annotations.

### AspGD and community interconnections

As a community-focused database, AspGD integrates and assembles information of various types in addition to genomic sequence and literature curation to improve and facilitate access to, and sharing of community resources. A recent and significant challenge for the *A. nidulans* research community was the simultaneous existence of two different versions of the genome annotation, both with their individual strengths. A major collaborative



**Figure 4.** Examples of modifications to *A. niger* gene models based on analysis with PASA. The changes include (A) extension of coding sequence (CDS), (B) update of internal exon structure and (C) gene merging. Rectangles represent gene exons and are colored according to the legend. Displayed with each structure diagram is the name of the gene modified by the PASA analysis or the GenBank accession number of each EST and full-length cDNA sequence supporting the modifications.

community effort produced the Eurofungbase annotation, in which individual experts conducted manual review and updating of genes with which they were familiar (10). In a parallel effort, researchers at the Broad Institute produced a genome-wide reannotation using modern computational tools. At AspGD, we made resolution of this situation a priority. We analyzed the differences and performed a systematic merge of the two sets, first retaining the careful manual efforts of the Eurofung team, and then integrating the updated computationally based gene model changes where they did not introduce a conflict with the manual curation. The Locus History that we provide for each gene provides details on all updates made, or information conflicts, which pertain to the annotation of the gene model.

A particularly valuable experimental resource for the *A. nidulans* community is the systematic *A. nidulans* gene knockout collection. Knockout cassettes are now available from the Fungal Genetics Stock Center (FGSC, [http://www.fgsc.net/Aspergillus/KO\\_Cassettes.htm](http://www.fgsc.net/Aspergillus/KO_Cassettes.htm)). The community asks that mutant strains generated with the cassettes be deposited with the FGSC as soon as possible after construction. At AspGD, we have added a ‘KO Cassette Available’ link to the External Resources section on the Locus Summary page of each gene for which this is the case, to facilitate utilization of this resource and generation of the strain collection.

New web-based community resources in AspGD include a page for sharing of laboratory methods and protocols, and a page for downloading of community-provided images and movies (<http://www.aspgd.org/Methods.shtml> and <http://www.aspgd.org/Images.shtml>, respectively). To contribute to either of these resources, or to suggest other pages that might be useful, please contact the AspGD curators at [aspergillus-curator@lists.stanford.edu](mailto:aspergillus-curator@lists.stanford.edu).

## SUMMARY/FUTURE DIRECTIONS

Going forward, we will curate the experimental data from the scientific literature on *A. niger* and then *A. oryzae*, as

decided by community consensus at the Asperfest meeting at the 10th European Conference on Fungal Genetics, in 2010. Subsequently, we will curate *A. flavus*, *A. clavatus*, *A. terreus* and *N. fischeri/A. fischerianus*. Each additional species will be curated and fully incorporated into AspGD alongside *A. nidulans* and *A. fumigatus*. We will also be further enhancing the web site and tools to be able to store and display sequence data for multiple strains of any given *Aspergillus* species.

Anticipating the wealth of large-scale data that are becoming available from the use of new high-throughput technologies, we are preparing to provide tools for interactive web-based visualization of these data. We are also incorporating RNA-Seq as an experimental input into the PASA genome annotation pipeline, to use this information alongside comparative data to improve the gene model annotations across all of the sequenced *Aspergillus* genomes.

We welcome and encourage all suggestions, comments and questions and can be reached by email at [aspergillus-curator@lists.stanford.edu](mailto:aspergillus-curator@lists.stanford.edu).

## ACKNOWLEDGEMENTS

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